

WHAT IS CLAIMED IS:

- 1 1. A method of detecting the presence of an immobilized molecular
2 analyte comprising:
 - 3 (i) contacting a molecular analyte immobilized on a molecular analyte solid
4 support with a film layer comprising a molecular ligand zone, wherein said molecular ligand
5 zone comprises a molecular ligand;
 - 6 (ii) wetting the molecular ligand and allowing the molecular ligand to
7 diffusibly migrate to a molecular ligand binding site of the molecular analyte to produce a
8 detectable product; and
 - 9 (iii) detecting said detectable product.
- 1 2. The method of claim 1, wherein:
 - 2 (i) said molecular analyte comprises a first component of a donor-acceptor
3 pair;
 - 4 (ii) said molecular ligand comprises a second component of said donor-
5 acceptor pair; and
 - 6 (iii) said detectable product is a complex between the molecular ligand and
7 the molecular analyte, wherein the position of the first component of the donor-acceptor pair
8 relative to the second component of the donor-acceptor pair allows detection of the complex.
- 1 3. The method of claim 2, wherein said molecular analyte is a nucleic
2 acid or a protein.
- 1 4. The method of claim 2, wherein said molecular ligand is a nucleic acid
2 or a protein.
- 1 5. The method of claim 1, wherein:
 - 2 (i) said molecular analyte comprises an enzyme;
 - 3 (ii) said molecular ligand binding site is an active site of the enzyme;
 - 4 (iii) said molecular ligand comprises an enzyme substrate; and
 - 5 (iv) said detectable product is the enzyme substrate after being catalyzed by
6 the enzyme.
- 1 6. The method of claim 1, wherein said molecular analyte is immobilized
2 on said molecular analyte solid support by binding said molecular analyte to a capture agent

3 immobilized on said molecular analyte solid support, wherein said binding is a molecular
4 analyte specific binding event.

1 7. The method of claim 6, wherein said capture agent is a protein or
2 nucleic acid.

1 8. The method of claim 1, wherein said film layer is a multilayered film
2 layer, wherein said multilayered film layer comprises the molecular ligand zone and at least
3 one additional zone, wherein said additional zone comprises a chemical or physical
4 environment that is unique within the film layer.

1 9. The method of claim 8, wherein said additional zone is below said
2 molecular ligand zone and is porous or water soluble.

1 10. The method of claim 1, wherein said hydrogel comprises acrylamide or
2 agarose.

1 11. The method of claim 10, wherein said semi-solid material is selected
2 from polyacrylamide and agarose.

1 12. The method of claim 1, wherein said film layer comprises at least two
2 molecular ligands, wherein said at least two molecular ligands are distributed in an array
3 format.

1 13. The method of claim 1, wherein said detectable product is detected by
2 mass spectrometry.

1 14. An apparatus comprising a molecular analyte layer and a film layer
2 wherein:

3 (i) the molecular analyte layer comprises a molecular analyte immobilized
4 on a molecular analyte solid support, wherein said molecular analyte comprises a molecular
5 ligand binding site; and

6 (ii) the film layer comprises a molecular ligand zone having a molecular
7 ligand, wherein, upon wetting of the molecular ligand zone, the molecular ligand can
8 diffusibly migrate to the molecular ligand binding site of the molecular analyte to produce a
9 detectable product.

- 1 **15.** The apparatus of claim 14, wherein:
2 (i) said molecular analyte comprises a first component of a donor-acceptor
3 pair;
4 (ii) said molecular ligand comprises a second component of said donor-
5 acceptor pair; and
6 (iii) said detectable product is a complex between the molecular ligand and
7 the molecular analyte, wherein the position of the first component of the donor-acceptor pair
8 relative to the second component of the donor-acceptor pair allows detection of the complex.
- 1 **16.** The apparatus of claim 15, wherein said molecular analyte is a nucleic
2 acid or a protein.
- 1 **17.** The apparatus of claim 15, wherein said molecular ligand is a nucleic
2 acid or a protein.
- 1 **18.** The apparatus of claim 14, wherein:
2 (i) said molecular analyte comprises an enzyme;
3 (ii) said molecular ligand binding site is an active site of the enzyme;
4 (iii) said molecular ligand comprises an enzyme substrate; and
5 (iv) said detectable product is the enzyme substrate after being catalyzed by
6 the enzyme.
- 1 **19.** The apparatus of claim 14, wherein said molecular analyte is
2 immobilized on said molecular analyte solid support by binding said molecular analyte to a
3 capture agent immobilized on said molecular analyte solid support, wherein said binding is a
4 molecular analyte specific binding event.
- 1 **20.** The apparatus of claim 19, wherein said capture agent is a protein or
2 nucleic acid.
- 1 **21.** The apparatus of claim 14, wherein said film layer is a multilayered
2 film layer, wherein said multilayered film layer comprises the molecular ligand zone and at
3 least one additional zone, wherein said additional zone comprises a chemical or physical
4 environment that is unique within the film layer.

1 **22.** The apparatus of claim **21**, wherein said additional zone is below said
2 molecular ligand zone and is porous or water soluble.

1 **23.** The apparatus of claim **14**, wherein said molecular ligand zone
2 comprises a molecular ligand within a hydrogel.

1 **24.** The apparatus of claim **23**, wherein said hydrogel comprises
2 acrylamide or agarose.

1 **25.** The apparatus of claim **14**, wherein said film layer comprises at least
2 two molecular ligands, wherein said at least two molecular ligands are distributed in an array
3 format.

1 **26.** The apparatus of claim **14**, wherein said detectable product is detected
2 by mass spectrometry.